Michigan Department of Community Health
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LabLink

Michigan Department of Community Health
Bureau of Laboratories

"Quality Laboratory Science for Healthier People and Communities"

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CDC Confirms Case of Swine Influenza A, H1N2 in a Michigan Resident

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In September 2007, MDCH confirmed influenza A (H1N2) in a specimen that had previously tested positive by a rapid test. Testing at MDCH included viral culture followed with H subtyping by indirect fluorescent antibody (IFA) and N subtyping by PCR. Because of the rarity of isolating an H1N2 virus, this isolate was submitted to CDC for confirmation.

The Centers for Disease Control and Prevention (CDC) confirmed the strain as A (H1N2). In addition, the strain was determined to be of swine origin. Testing at CDC included real-time PCR to confirm subtyping followed by complete genome sequencing and viral culture for additional viral characterization.

Results to date have determined this virus to be a triple reassortant virus meaning that the virus has genes inherited from human viruses, from classical swine viruses and from unknown avian species. These triple reassortant viruses have been circulating in pigs in the U.S. for several years. They appeared as a result of reassortment, most likely in pigs, since pigs have receptors that can bind human, avian and influenza viruses. There is no indication that this reassortment occurred in the infected individual.

This unusual virus was isolated from a 16-month-old child who developed moderate flu-like symptoms and subsequently recovered with no complications. A comprehensive follow-up investigation by MDCH, CDC and the local health department is currently underway. No additional cases have been identified at this time, and this case is believed to be an isolated event with the most probable exposure route being contact with infected swine.

This case highlights many important aspects of increased off-season influenza surveillance to detect circulating human and novel influenza viruses. During the off-season when prevalence of influenza in the community is low, the positive predictive value of influenza rapid tests decreases. MDCH suggests that positive rapid tests during low influenza activity be confirmed by viral culture at the MDCH lab. Other important aspects this case demonstrates, besides the value of off-season surveillance, are the importance of pandemic/novel influenza preparedness for the state lab and its surveillance partners, and the tremendously important role played by surveillance partners in these activities.

Newborn Screening for Cystic Fibrosis Begins October 1, 2007

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Cystic fibrosis (CF) is one of the most common autosomal recessive genetic disorders affecting children. It occurs in about 1 in 3,500 Caucasian newborn infants, with a lower incidence in other ethnic groups. The most common and most severe genetic mutation causing cystic fibrosis is a three-basepair deletion (F508) in the cystic fibrosis transmembrane regulator (CFTR) gene. More than 1500 mutations in the CFTR gene have now been reported (http://www.genet.sickkids.on.ca/cftr/app).

The objective of newborn screening for CF is to identify most affected children and make early referral to a specialty center accredited by the national Cystic Fibrosis Foundation that offers aggressive intervention to allow normal growth and delay the onset of lung disease. Delays in diagnosis can result in irreversible bronchiectatic changes with dramatic impact on the lungs' vital functions.

The pancreatic enzyme immunoreactive trypsinogen (IRT) is usually elevated at birth when pulmonary symptoms are not evident. . The Michigan Department of Community Health (MDCH) Newborn Screening Laboratory as the first tier in screening will use IRT concentration. Testing is performed using a solid phase 2 site fluoroimmunometric assay (Perkin Elmer Life Sciences, Inc.).

Specimens with elevated values (the highest 4%) for IRT will be followed up by DNA mutation assay (Third Wave Invader Technology) using a panel of 42 mutations as a second tier screen. Both tiers of the screening test for CF can be run on the dried filter paper blood spots collected. Specimen collection will not change with the addition of CF (i.e., additional samples will not be needed).

MDCH will report a positive screening result for CF when:

- One or two CFTR mutations are present
- IRT levels are =99.8% and no CFTR mutations are present.

It is anticipated that about 350 Michigan newborns will have a positive CF screen each year. **A positive screen does not necessarily mean a child has CF**. Approximately **1 in 10** of these infants will have a diagnosis of CF. The majority of the others will be carriers of CF and will not have symptoms of the disease. Infants with a positive newborn screen will require confirmatory sweat chloride testing at a Cystic Fibrosis Center. More extensive genetic testing may be needed. Genetic counselors will be available through the CF Center for patients undergoing confirmatory testing, as well as for those identified as carriers. For children with a confirmed diagnosis of CF, ongoing assessment and treatment at a CF Center is recommended. There are currently five accredited CF Centers in Michigan:

- University of Michigan (Ann Arbor), serves as the state's CF NBS Coordinating Center, *Phone: (734) 764-4123*
- Children's Hospital of Michigan (Detroit), *Phone: (313) 745-5541*
- DeVos Children's Hospital (Grand Rapids), *Phone: (616) 391-2125*
- MSU Kalamazoo Center for Medical Studies (Kalamazoo), *Phone: (269) 337-6433*
- Michigan State University Cystic Fibrosis Center (Lansing), *Phone: (517) 364-5440*

Prompt communication and interpretation of results with the patient's primary care physician will be provided by MDCH and CF Coordinating Center staff. If you have any questions or concerns, please do not hesitate to contact us at (517) 335-9205 or by e-mail at mdch-newbornscreening@michigan.gov.

Salmonella Serotyping 2003-2007

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Enteric/STD/Chromatography Unit

Non-typhoid *Salmonella* sp. usually causes gastrointestinal infections accompanied by diarrhea, fever and abdominal cramps. It can cause localized infections or even bacteremia in some individuals, particularly in immunocompromised patients. There are 2,500 recognized serotypes of *Salmonella*[1].

Typically, the top two serotypes (Enteritidis and Typhimurium) account for approximately 40% of the strains received in the MDCH laboratory. The remaining top ten-ranked Salmonella change annually due to outbreaks (see Table 1). The top ten serotypes normally account for 70-75% of the strains received. Serotyping *Salmonella* strains helps to identify outbreaks at an early stage. Further DNA fingerprinting using pulse field gel electrophoresis (PFGE) allows for identification of related strains of the same serotype.

Table 1. Predominant *Salmonella* serotypes isolated in the last five years

2003	2004	2005	2006	2007
ENTERITIDIS 146 (17.5%)	ENTERITIDIS 185 (21.8%)	ENTERITIDIS 214 (21.2%)	ENTERITIDIS 198 (19.1%)	ENTERITIDIS 210 (21.8%)
TYPHIMURIUM 122 (14.6%)	TYPHIMURIUM 152 (17.9%)	TYPHIMURIUM 188 (18.7%)	TYPHIMURIUM 181 (17.4%)	TYPHIMURIUM 173 (17.9%)
NEWPORT 80 (9.6%)	HEIDELBERG 64 (7.5%)	HEIDELBERG 69 (6.8%)	4,5,12:i:- 86 (8.3%)	4,5,12:i:- 69 (7.2%)
HEIDELBERG 58 (7.0%)	NEWPORT 61 (7.2%)	NEWPORT 68 (6.7%)	HEIDELBERG 55 (5.3%)	NEWPORT 56 (5.8%)
ORANIENBURG 27 (3.2%)	JAVIANA 22 (2.6%)	ORANIENBURG 36 (3.6%)	NEWPORT 53 (5.1%)	HEIDELBERG 52 (5.4%)
SAINTPAUL 27 (3.2%)	PARATYPHI B 19 (2.2%)	SAINTPAUL 23 (2.3%)	BRAENDERUP 48 (4.6%)	THOMPSON 26 (2.7%)
THOMPSON 25 (3.0%)	SAINTPAUL 17 (2.0%)	MONTEVIDEO 16 (1.6%)	ORANIENBURG 20 (1.9%)	TENNESSEE 22 (2.3%)
MUENCHEN 17 (2.0%)	BERTA 16 (1.9%)	SCHWARZENGRUND 16 (1.6%)	4,5,12:b:- 20 (1.9%)	4,5,12:b:- 19 (2%)
AGONA 16 (1.9%)	ORANIENBURG 16 (1.9%)	STANLEY 14 (1.4%)	THOMPSON 19 (1.8%)	**ORANIENBURG 18 (1.9%)
HADAR 16 (1.9%)	INFANTIS 15 (1.8%)	AGONA 11 (1.1%)	*INFANTIS 18 (1.7%)	**PARATYPHI B 18 (1.9%)

* During fiscal year 2006, there were also 18 *Salmonella* serotype Montevideo, Saintpaul and Group D 9,46:a:- isolates.

**During fiscal year 2007, there were also 18 *Salmonella* serotype Stanley isolates.

2003 – 833 strains identified from 78 serotypes
2004 – 850 strains identified from 81 serotypes
2005 - 1008 strains identified from 87 serotypes
2006 – 1038 strains identified from 84 serotypes
2007 – 964 strains were identified from 86 serotypes

[1] Bopp, C. A., Brenner F. W., Fields, P. I., Wells, J. G., and Stockbine, N. A., "Escherichia, Shigella, and Salmonella", *Manual of Clinical Microbiology*, Eighth Edition, 2003, ASM Press, Washington, D.C., pp. 654-671.

Rabies Submissions Exploding

Patty Clark, M.P.H.
Viral Serology/Viral Isolation/Viral Molecular Unit

To date this year, the Michigan Department of Community Health Bureau of Laboratories has received a record number of specimens for rabies testing. As of October 6, 2007, the lab has received 3323 rabies specimens. This is the highest number of specimens received in any year since the lab began rabies testing (data going back to 1954). In 2006, 2382 rabies specimens were received in the entire year. The majority of the submissions in most years are received from June through mid-September. This year was no exception. The week with the largest total number of rabies submissions was the week ending September 1, when 352 rabies specimens were received. In 2006, the largest number of rabies specimen submissions occurred one week earlier, the week ending August 25, with a total of 190 specimens received. This is an 85% increase in specimen submissions for this one week only. From January through October 6, 2007 the lab had a 39.5% increase in rabies submissions when compared to the same time period in 2006.

The number of rabies positives detected is also up dramatically. In 2006, the lab detected a total of 49 rabies positive animals out of 2869, an overall rabies positivity rate of 1.7%, with a positivity rate from January through October 6 of 1.8%. From January through October 6 this year, the lab detected 198 positives out of 3266 submissions for a rabies positivity rate of 6.1%. Typically the annual rabies positivity rate of between 1-5%. A breakdown of positives by species and county for 2006 and 2007 can be found in the attached maps.

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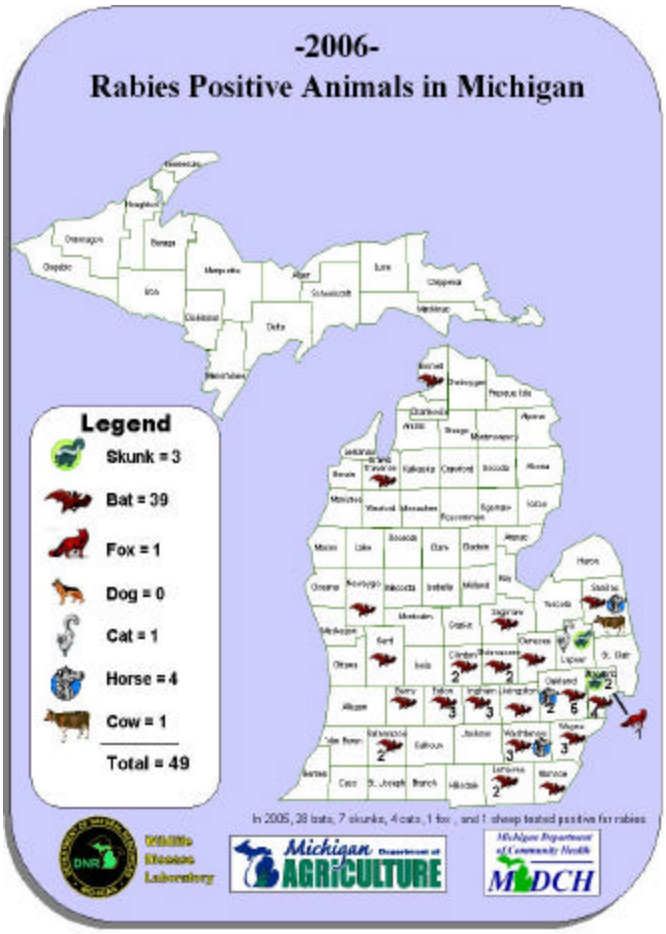
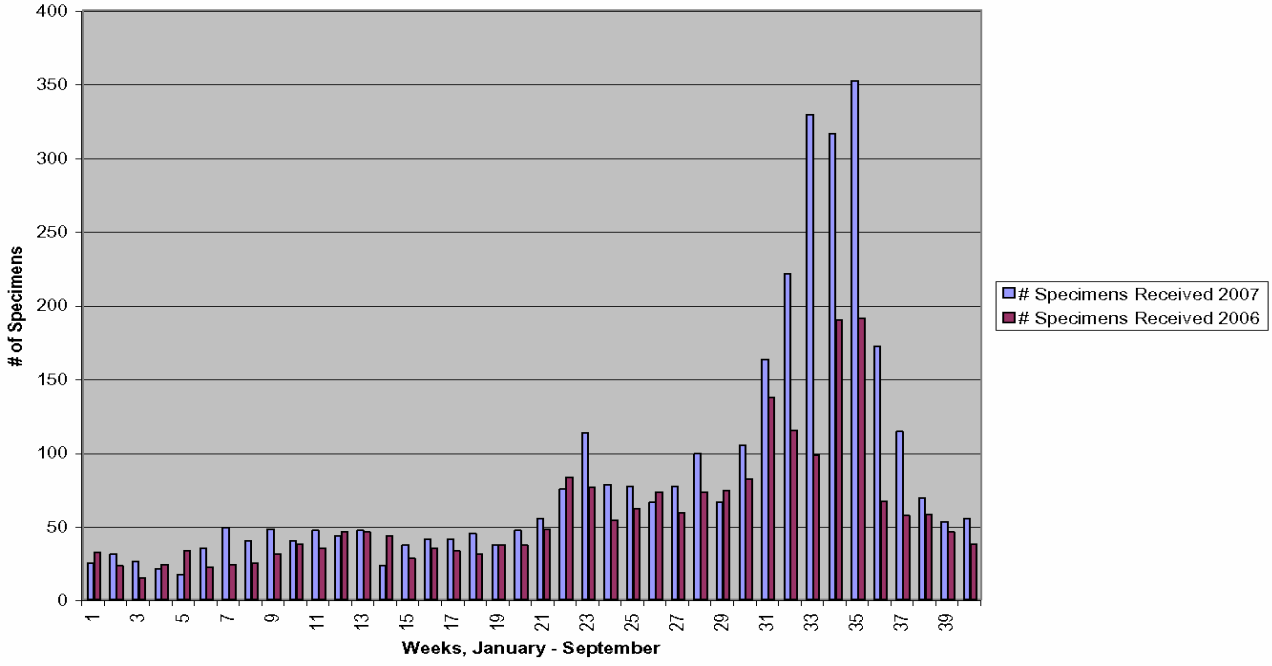
The reason for the record number of submissions and positives is not clear. There has been more publicity in newsprint and television news surrounding rabies positive bats this year. This publicity may be causing the increase in submissions, which in turns results in an increase in detecting positives. An alternate reason may have to do with the bat population in Michigan. It is possible the bat population has increased resulting in either more contact between bats and people and their pets or more disease in the bat population. At this point it is merely speculation as there is no data to confirm either hypothesis.



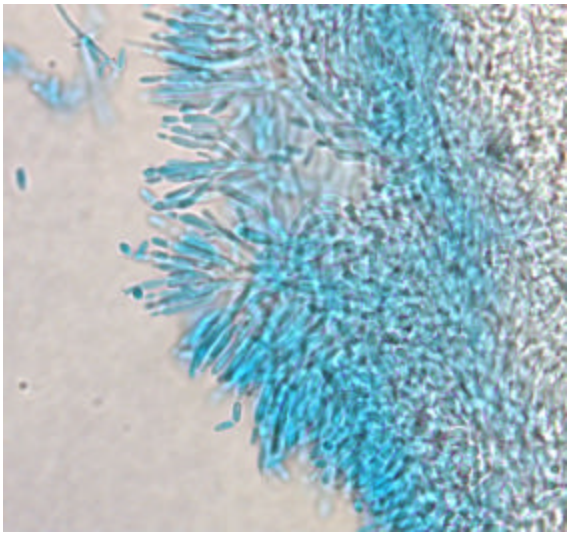
Rabies Specimens Received Per Week, Jan - September



FUN FUNGI.....
Myrothecium species

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

Last Issues Picture Quiz Answer:



***Myrothecium* species**

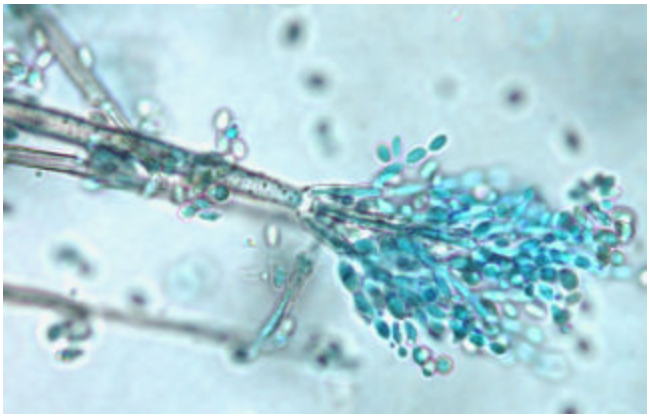
Myrothecium species are common soil saprophytes. Some species have been found to be plant parasites, causing leaf spot on various plants. They have a worldwide distribution. There are currently 13 recognized species.

Myrothecium species are typically velvety, white to rosy-buff and form dark green to black sporodochia. These sporodochia may be flat, discoid, or form on short stalks. Conidiophores are hyaline, branched and formed in compact brush-like clusters. Long phialides form at the ends of the conidiophores and produce ovoid, fusoid, or cylindrical conidia that are hyaline to dark green in color. These dark green conidia (phialospores) form in loose columns or in slimy masses. *Myrothecium* species are typified by the formation of dark green conidia in slimy masses on sporodochia.

References:

1. Baron, George. 1977. *The genera of Hyphomycetes from Soil*. Robert Krieger Publishing Co. Huntington, N.Y.
2. Domsch, K.H., Gams, W., Anderson, T. 1993, *Compendium of Soil Fungi*. IHW-Verlag, Germany.

This Issues Picture Quiz: What Mould is this?



Chemical Terrorism/ Exposure Training Available Online!

Martha Boehme, MT(ASCP)
Ninah Sasy, MT
Division of Chemistry and Toxicology

There are unique requirements for collecting specimens from victims of an intentional chemical exposure event. MDCH continues to offer training for hospital staff in the proper collection and handling of these specimens. Martha Boehme, the Chemical Terrorism Laboratory Educator, conducts this training, "Laboratory Response and Hospital Preparedness in a Chemical Exposure (Terrorism) Event." This training can be scheduled as a live session or viewed as a self-paced online training.

Please visit www.michigan.gov/mdchlab > Chemistry & Toxicology> Chemical Terrorism Laboratory Preparedness for more information about the course.

To schedule a session at your hospital, please call 517-335-9654. To register for the on-line training, go to http://mi.train.org

The Michigan Nurses Association, an accredited approver of continuing nursing education by the American Nurses Credentialing Center Commission on Accreditation, approves "Laboratory Response and Hospital Preparedness in a Chemical Exposure Event" for 1.0 contact hours. The presenter and the planning team have no commercial interests or conflicts of interest to disclose. No commercial support has been received for this event.

Improved Turn Around Times for Newborn Screening Program

The Newborn Screening Program has improved turn around time for both the delivery of specimens and lab reports.

In addition to hospital based couriers, arrangements have been made to have Quest Diagnostics and the United Parcel Service transport samples to the laboratory overnight and in time to be tested the next day. New hospitals have been systematically added and now more than sixty percent of samples arrive via overnight delivery.

The Michigan Historical Museum Summer Camp

Teresa Miller

The Michigan Historical Museum sponsored their annual Summer Sizzles Day Camp. The camp has been a success for the past eight years offering a variety of programs for children ages 9 through 16 years old.

This year, the Camp included a three-hour session called **"*Keeping Michigan Healthy.*"** a tribute to the Bureau of Laboratories' Centennial Celebration. On August 15, 2007, the Bureau presented a session for children 13-16 years of age that detailed historical events, which had significant impact on the Bureau's work and the Michigan community.

A team of BOL employees prepared the events for the session. Christin Lott, Paul Loconto, Martha Boehme, and Ninah Sasy represented the Chemistry and Toxicology Division. Lott gave a power point presentation detailing the events of the 1973 PBB incident in Michigan. All of the team members participated in the hands on activity, which re-enacted this tragic chemical mishap.

Patricia Clark, Donna Huntzinger, and Teresa Miller represented the Infectious Disease Division. Clark gave a power point presentation highlighting an historical account of microbial methodologies for DNA/RNA at the Bureau of Laboratories. This presentation also introduced general information about the differences between DNA and RNA, bacteria and viruses, and an historical synopsis of norovirus activity. For an exciting hands-on activity, students were aided in performing the procedure for DNA extraction from their own cells. Extracted DNA was placed in an amulet necklace. The participants proudly displayed their DNA necklaces during the remainder of the camp session. Each participant was allowed to take the DNA necklace home.

This camp session was a huge success. Seventeen children attended and they all thoroughly enjoyed the hands-on activities.

We would like to extend our gratitude for the support given by the Michigan Historical Museum Educational Staff. Jo Anne Arasim, from education development, was appreciative of the quality of the programs offered.

From the Volunteer's Corner:

The Wonder of It All

Jim Armelagos, M.S.
Quality Control Unit

On Saturday, August 18, 2007 the Impression Five Science Center in Lansing hosted an event called, "HealthWorks" sponsored by the Capital Healthcare and Employment Council of the Capital Area Michigan Works! program. The goal was to provide the public who were attending this free "Super Saturday" event at the Science Center an introduction to other health care job opportunities other than becoming a physician or a nurse. I and other health professionals, who could possibly encourage fellow citizens to think about having future careers in the health field, were recruited to present.

Each health care profession developed a hands-on experiment. The clinical lab demonstration was intended to show how a large majority of microbes in this world work wonders within our bodies and all around us. Without these microscopic friends, we could not survive and that there is no need to stop the growth of this hidden microbial world around and inside us. Additionally the participants were inspired to become a laboratory science professional and have some fun.

Set up for the activity required preparation of materials: a microbial culture obtained from whole milk held at room temperature (RT) for 48 hours, a general growth medium as blood agar plates, dilution blanks, inoculating cotton-tipped applicators, ½ inch blank disks, pipettes, forceps, flasks of sterile purified H_2O (the control agent), an antibacterial soap (the first inhibiting agent) and some household bleach (the second inhibiting agent). All these items were used to simulate a minimum inhibition concentration (MIC)-like procedure. These tools would help us bring to light the hidden microbial world which is easily thought of by many as dark and dangerous and not needed by us macro-creatures.

Sixty to seventy wondering participants, ages ranging from five to 75 years attended the classroom setting for 30-minute hands-on experimental presentations. Each session held around 15 rookie experimenters and began with the general background, techniques used and what to expect once the inoculated blood agar plates were taken out of the laboratory area and transported back to their individual home for incubation. "Wow! Mom and Dad our home is going to become a science laboratory!"

There were wonderful questions, which covered things like why we like to do our jobs, why we had chosen the line of work we have and what type of schooling it took to become the professionals that we are today. Most of these questions came directly from the older students. The younger students wondered how long before they would see something grow/appear; how long before the now hidden becomes larger than what the literally nothing that they were currently seeing on that 'bloody' agar surface. "I want to see what's going to gross out my sister/brother, now!"

Despite the procedural mishaps of pushing into the agar surface too deeply with the sterile swabs, contaminating sterile materials and putting the sterile disks too close together so as to cause cross-over of chemical inhibitory agents, the patient museum volunteers wanted to try out their humble skills, take the experimental ingredients provided and spend some time in wonder of it all.

Lyme Disease, A Case Study

William Crafts MT, ASCP
Bacterial and Parasitic Serology Unit

The serologic diagnosis of Lyme borreliosis (LB), especially early disease, continues to present challenges for physicians and laboratorians. Variations in *Borrelia burgdorferi* strains utilized in commercial and in-house enzyme immunoassays (EIA) and western blot assays (WB) and interpretation differences contribute to this problem.

Lyme disease, now referred to as Lyme Borreliosis (LB), is the most common vector-borne disease in North America and Europe and represents a public health concern. *Borrelia burgdorferi* sensu lato group represents approximately 11 genospecies but only three are pathogenic to humans. *B. burgdorferi* sensu stricto is the only pathogen in this group endemic to North America. *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* are European pathogens. Since 1982, more than 200,000 LB cases in the United States have been reported to CDC and approximately 20,000 new cases are reported annually. In Europe, LB surveillance numbers are difficult to establish due to reporting differences among countries but are estimated to occur approximately 80,000 new cases annually. The current case definition for surveillance purposes includes either physician-diagnosed EM lesions = 5 cm in diameter or at least one latent symptom (arthritis, neurologic, or cardiac) and reactive Lyme serology.

On July 24, 2007, Michigan Department of Community Health (MDCH) received serum on a 4-year-old male for LB testing. The following information was provided on the test requisition; date of collection 7/17/07, rheumatologic symptoms were first noted on 6/1/07, and the county of exposure was northwest lower Michigan.

The whole-cell sonicated *B. burgdorferi* B31 strain EIA result was positive and the IgG WB result was negative. Since the EIA index was three times the cutoff (index value 3.41) a call was made to the submitter and physician to obtain additional information. The patient resides in Germany in an area endemic for LB and arrived in the U.S. on July 5th. The physician noted a slight fever and a 12 x 5 cm erythematous target lesion on the child's chest. Based on this information, an IgM WB assay was performed at MDCH and found to be negative. The serum specimen was forwarded to the manufacturer of the western blot kit to rule out European LB.

A 3rd generation EIA designed for use in Europe utilizes purified *B. burgdorferi* B31 strain, *B. afzelii* PKO strain and *B. garinii* G2 strain was performed. The IgM result was equivocal and the IgG was positive. A 2nd generation WB, which utilizes partially purified antigens of *B. afzelii* PKO and purified OspC (outer surface protein) of *B. garinii* G2 strain, was IgM positive (41 and 23 kDa bands) and IgG positive (41, 23 and 14 bands). Based on EIA and WB results, the patient was diagnosed with a recent exposure to European *Borrelia* spp.

The original information provided to MDCH indicated late stage disease because of rheumatologic symptoms and length of time between date of onset and date of collection greater than 30 days. According to current CDC guidelines, only IgG WB testing is recommended for diagnosis of late stage LB. However, based on additional information provided by the physician, an IgM WB was performed. Although the result was negative an unusual band was observed. Band 37 kDa, referred to as FlaA or Arp (arthritis related-protein), is a possible indication of early LB but its specificity has not been established. The manufacturer of the WB kit used at MDCH reported that the strain utilized in the nitrocellulose immunoblot strips did not contain

B. afzelii and *B. garinii* strains responsible for European LB.

LB cases were defined as patients exhibiting EM rash, supportive clinical signs/symptoms, travel history to endemic locations, evidence of tick bite, and laboratory confirmation. Retrospective analysis of LB cases over the past seven years at MDCH show a high correlation between lyme EIA index values = 2.0 and positive WB results.

Obtaining detailed clinical information and travel history, especially on patients with lyme EIA index values = 2.0, will assist in the diagnosis of LB. This case outlines the significance of providing detailed clinical symptoms and travel history to enhance diagnostic capabilities.

References:

1. Aguero-Rosenfeld, M. E., Wang, G., Schwartz, I. and Wormser, G. P. 2005. Diagnosis of Lyme Borreliosis. *Clin. Microbiol. Reviews* , 18.3:484-509.

2. Goodman, J. E., Dennis, D. T. and Sonenshine, D. E. (eds.) 2005. Lyme Borreliosis IN: Tick-Borne Diseases of Humans. pg. 176-206.

Bureau of Laboratories Vision

The Bureau of Laboratories is a stronger, more diverse team within an integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

Bureau of Laboratories Mission

We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

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